SENSITIVITY ASSESSMENT OF MOLECULAR TOOLS FOR DETECTION OF RICKETTSIAE

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PROGRESS REPORT

Rickettsioses are febrile illnesses caused by obligate intracellular gram-negative bacteria. These diseases are clinically hard to diagnose due to their non-specific signs and symptoms. Laboratory confirmation is required for any definite diagnosis. Although Indirect Fluorescent Assay is widely used to detect rickettsial infections it is limited in regards to early detection and species identification. Aiming to better understand the epidemiology of pathogenic rickettsiae in Thailand, molecular methods have been utilized to overcome the limitations of traditional serological methods in order to identify the specific species of *Rickettsia* agents in the specimens more accurately. Our report on the approaches used for the assessment of sensitivity and specificity of these molecular tools is as follows. A portion of four rickettsial specific genes, 17 kDa, citrate synthase, *rompA* and 56 kDa were PCR amplified and subsequently cloned into TA cloning vector. The resulting constructs, pJG1, pJG2, pJG3 and pJG4 were then transformed into *Escherichia coli* for further propagation. The purified plasmids were serially diluted and used as copy number controls for the sensitivity assessment. Our molecular tools are able to detect approximately 10-10^3 rickettsial molecules in the specimen with 100% specificity. Furthermore, the engineered rickettsial constructs can be unlimitedly produced and used as controls in PCR substituting the use of purified rickettsial genomic DNA. This work is part of AFRIMS laboratory capability enhancing plan to qualify for the national rickettsiae reference laboratory.

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